

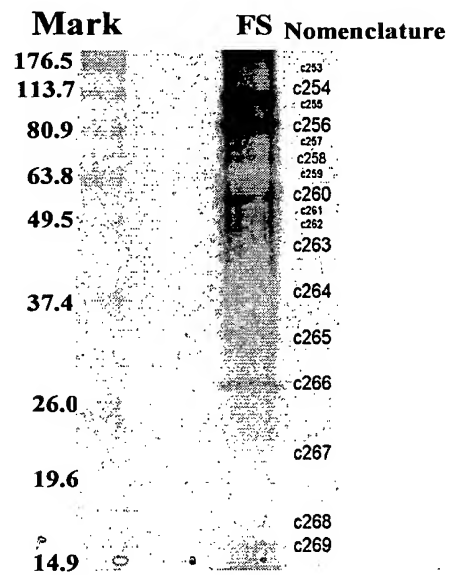
Figure 1. Isolation of human Fibrous Sheath**Figure 2. SDS-PAGE of human fibrous sheath proteins**

Figure 3. Microsequencing of Fibrous Sheath Proteins

Each band of fibrous sheath protein was microsequenced by mass spectrometry. The sequence result was summarized in table 1. The band C265 was identified as an unknown protein (DKFZp434N1235). Peptides microsequenced from the C265 band are indicated by bold.

Microsequencing of C265 by Mass Spectrometry

mhrepakkka ekrlfdassf **gkdllaggva** aavsktavap iervklllqv qasskqisp
 arykgmvdcl vripreqgff sfwrgnlanv iryfptqaln fafkdkykql fmsgvnkekq
 fwrwflanla sggaagatsl cvvypldfar trlgvdigkg peerqfkglg dcimkiaksd
 giaglyqgfg vsvqgiivyr **asyfgaydtv** **kgllpkpkkt** pflvsffiaq vvtcsgils
 ypfdtvrrrm mmqsgeakrq **ykgtdcfvk** **iyqhegissf** frgafsnvlr gtggalvlvl
 ydkikeffhi diggr

Mouse Orthologues of SFEC

msnesskkqs skkalldpvs fskdllaggv aaavskttva piervklllq vqasskqisp
 earykgmldc lvripreqgf lsywrgnlan viryfptqal nfafdkkyke lfmsgvnkek
 qfwrwflanl asggaagats lcvvypldfa rtrlgvdigk gpeqrqftgl gdcimkiaks
 dgliglyqgf gvsvqgiivy rasyfgaydt vkgllpkpke tpflvsfiia qivttcsgil
 sypfdtvrrr mmmqsgesdr qykgtdcfl kiyrhegvpa ffrgafsnil rgtggalvlv
 lydkikefln idvgsssgd

Figure 4. SFEC is a testis specific Protein- Northern Analysis

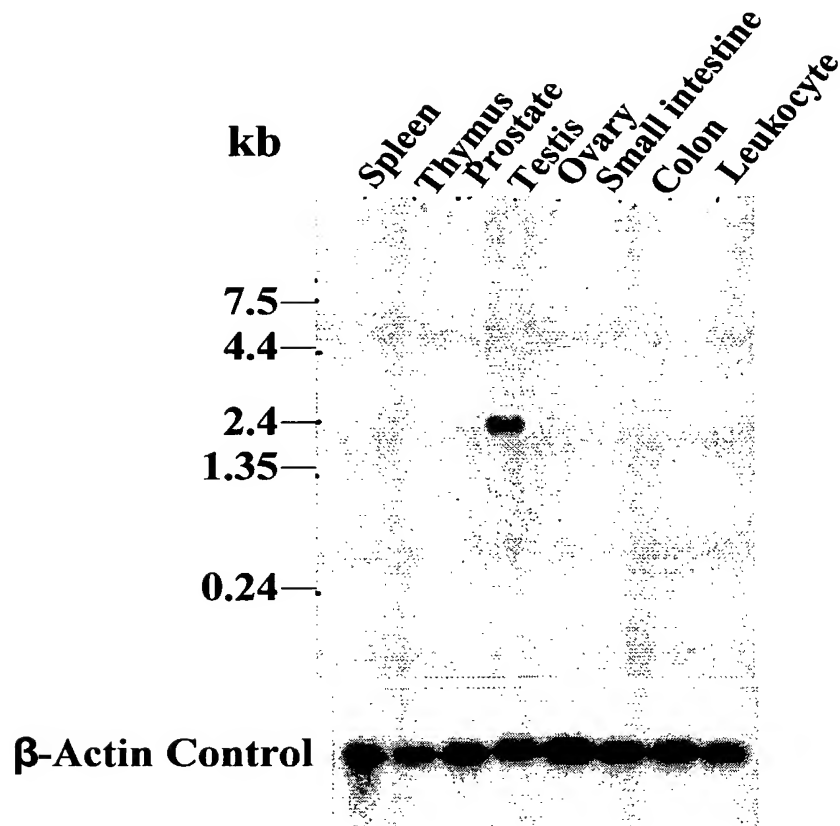


Figure 5- Dot blot analysis (upper panel) and human MTE array (lower panel) demonstrating testis specific expression of SFEC

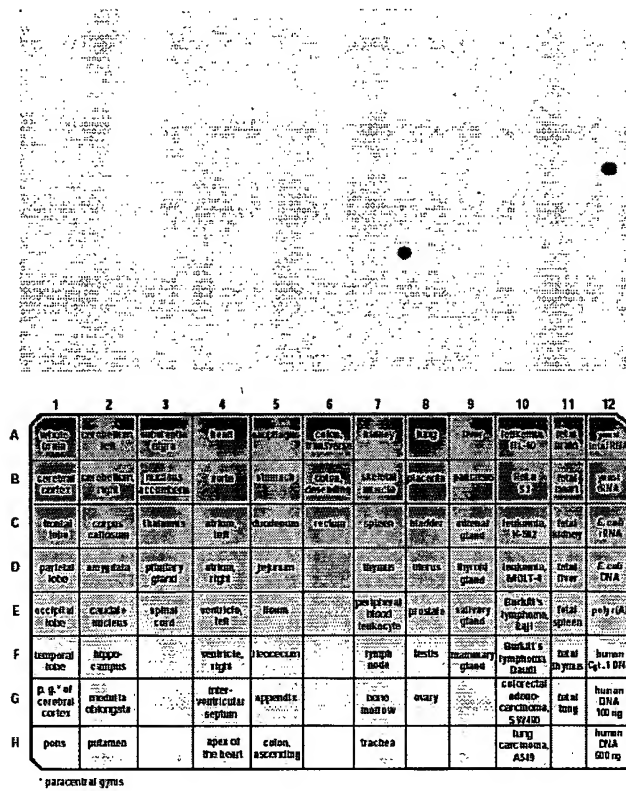
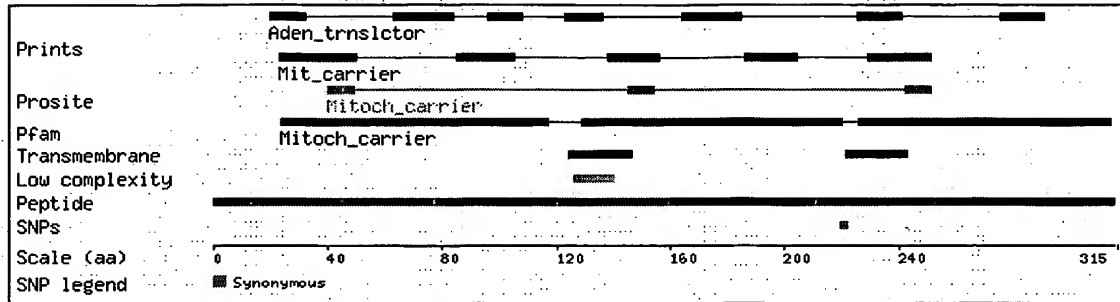


Figure 6- Functional Domains of SFEC

| Domain type | Accession number | Description | Start | End |
|-------------|------------------|----------------|-------|-----|
| prints | PR00926 | Mit_carrier | 23 | 36 |
| prints | PR00926 | Mit_carrier | 36 | 50 |
| prints | PR00926 | Mit_carrier | 85 | 105 |
| prints | PR00926 | Mit_carrier | 138 | 156 |
| prints | PR00926 | Mit_carrier | 186 | 204 |
| prints | PR00926 | Mit_carrier | 229 | 251 |
| prints | PR00927 | Aden_tnsictor | 20 | 32 |
| prints | PR00927 | Aden_tnsictor | 63 | 84 |
| prints | PR00927 | Aden_tnsictor | 96 | 108 |
| prints | PR00927 | Aden_tnsictor | 123 | 136 |
| prints | PR00927 | Aden_tnsictor | 164 | 185 |
| prints | PR00927 | Aden_tnsictor | 225 | 241 |
| prints | PR00927 | Aden_tnsictor | 275 | 290 |
| Pfam | PF00153 | Mitoch_carrier | 24 | 117 |
| Pfam | PF00153 | Mitoch_carrier | 129 | 220 |
| Pfam | PF00153 | Mitoch_carrier | 226 | 314 |
| prosite | PS00215 | Mitoch_carrier | 40 | 49 |
| prosite | PS00215 | Mitoch_carrier | 145 | 154 |
| prosite | PS00215 | Mitoch_carrier | 242 | 251 |

| Domain type | Start | End |
|----------------|-------|-----|
| Transmembrane | 124 | 146 |
| Transmembrane | 221 | 243 |
| Low complexity | 126 | 140 |

Figure 7. Alignment of Amino Acid Sequences of SFEC with other human proteins having a similar domains

CLUSTAL W (1.74) multiple sequence alignment

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unk|VIRT5947|Blast_submission      MHREPAKKKAIEKRLFDASSFGKDLLAGGVAAAVSKTAVAPIERVKLLQV
tr|Q9HOC2                          MHREPAKKKAIEKRLFDASSFGKDLLAGGVAAAVSKTAVAPIERVKLLQV
sp|P12235|ADT1_HUMAN              -----MGDHAWSFLKDFLAGGVAAAVSKTAVAPIERVKLLQV
sp|P12236|ADT3_HUMAN              -----MTEQAISFAKDFLAGGIAAAISKTAVAPIERVKLLQV
sp|P05141|ADT2_HUMAN              -----MTDAAVSFAKDFLAGGVAAAVSKTAVAPIERVKLLQV
                                   *  *  *:****:***:*****

unk|VIRT5947|Blast_submission      QASSKQISPEARYKGNVDCLVRIPREQGFFSFWRGNLANVIRYFPTQALN
tr|Q9HOC2                          QASSKQISPEARYKGNVDCLVRIPREQGFFSFWRGNLANVIRYFPTQALN
sp|P12235|ADT1_HUMAN              QHASKQISAEKQYKGIIDCVVRIPKEQGFLSFWRGNLANVIRYFPTQALN
sp|P12236|ADT3_HUMAN              QHASKQIAADKQYKGIIDCVVRIPKEQGVLSFWRGNLANVIRYFPTQALN
sp|P05141|ADT2_HUMAN              QHASKQITADKQYKGIIDCVVRIPKEQGVLSFWRGNLANVIRYFPTQALN
                                   * :****:.. :***:***:****:***:*****

unk|VIRT5947|Blast_submission      FAFKDKYKQLFMSGVNKEKQFWRUFLANLASGGAAGATSLCVVYPLDFAR
tr|Q9HOC2                          FAFKDKYKQLFMSGVNKEKQFWRUFLANLASGGAAGATSLCVVYPLDFAR
sp|P12235|ADT1_HUMAN              FAFKDKYKQLFLGGVDRHKQFWRUFLANLASGGAAGATSLCFVYPLDFAR
sp|P12236|ADT3_HUMAN              FAFKDKYKQIFLGGVDKHTQFWRUFLANLASGGAAGATSLCFVYPLDFAR
sp|P05141|ADT2_HUMAN              FAFKDKYKQIFLGGVDKRTQFWRUFLANLASGGAAGATSLCFVYPLDFAR
                                   *****:*. :***:.. *****: * *****

unk|VIRT5947|Blast_submission      TRLGVDIGKGPEERQFQKGLGDCIMKIAKSDGIAGLYQGFVSVQGIIVYR
tr|Q9HOC2                          TRLGVDIGKGPEERQFQKGLGDCIMKIAKSDGIAGLYQGFVSVQGIIVYR
sp|P12235|ADT1_HUMAN              TRLAADVKGGAQREFHGLGDCIIFKSDGLRGLYQGFVSVQGIIVYR
sp|P12236|ADT3_HUMAN              TRLAADVKGSGTEREFHGLGDCIVKITSKGIRGLYQGFVSVQGIIVYR
sp|P05141|ADT2_HUMAN              TRLAADVKGKAGAREFERHGLGDCIVKITSKGIRGLYQGFVSVQGIIVYR
                                   ***.:*. :*:*****:*** *****: *****

unk|VIRT5947|Blast_submission      ASYFGAYDVTVKGLLPKPKKTPFLVSFFIAQVVTTCSGILSYFPDTRRRM
tr|Q9HOC2                          ASYFGAYDVTVKGLLPKPKKTPFLVSFFIAQVVTTCSGILSYFPDTRRRM
sp|P12235|ADT1_HUMAN              AAYFGVYDTAKGHLDPKPNVHIFVSWHIAQSVTAVAGLVSYFPDTRRRM
sp|P12236|ADT3_HUMAN              AAYFGVYDTAKGHLDPKPNTHIVVSWHIAQSVTAVAGLVSYFPDTRRRM
sp|P05141|ADT2_HUMAN              AAYFGIYDTAKGHLDPKPNTHIVSWHIAQSVTAVAGLVSYFPDTRRRM
                                   *:*** ***:***:***:.. :. :*** ***: ***: *****

unk|VIRT5947|Blast_submission      MMQSGE--AKRQYKGTLDGCVKIYQHEGISSFFRGAFSNVLRGTGGALVL
tr|Q9HOC2                          MMQSGE--AKRQYKGTLDGCVKIYQHEGISSFFRGAFSNVLRGTGGALVL
sp|P12235|ADT1_HUMAN              MMQSGRKGADIMYTGTVDCWRKIAKDEGAKAFFKGAUSNVLRGHHGGAFLV
sp|P12236|ADT3_HUMAN              MMQSGRKGADIMYTGTVDCWRKIFRDEGGAFFKGAUSNVLRGHHGGAFLV
sp|P05141|ADT2_HUMAN              MMQSGRKGTDIMYTGTVDCWRKIADEGGAFFKGAUSNVLRGHHGGAFLV
                                   *****:.. :. :***:***: ** :.*** :***:***** *****

unk|VIRT5947|Blast_submission      VLYDKIKEFFHIDIGGR
tr|Q9HOC2                          VLYDKIKEFFHIDIGGR
sp|P12235|ADT1_HUMAN              VLYDEIKKYV-----
sp|P12236|ADT3_HUMAN              VLYDELKKVI-----
sp|P05141|ADT2_HUMAN              VLYDEIKKYT-----
                                   *****:

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Heart (ADT 1): identity (69%) similarity (79%)

Liver (ADT 3): identity (67%), similarity (80%)

Fibroblast (ADT 2): identity (67%), similarity (79%)

Figure 8. Human Fibrous Sheath Peptides involved in Energy Production

| Gene/Gene Symbol | Peptides | Gene Locus | Tissue Distribution |
|--|--|-------------------|---|
| Aldolase A, Fructose- biphosphate ALDOA | 1)GILAADESTGSIK 2)IGEHTPSALAIMENANVLAR 3)GVVPLAGTNGETTTQGLDGLSER 4)FSHEEIAMATVTALR 5)IGEHTPSALAIMENANVLAR | 16q22-q24 | Ubiquitously expressed |
| Pyruvate Kinase PKM2, Unkown protein | 1)NTGIICTIGPASR 2)GADFLVTEVENGGSLGSK 3)GVNLPGAAVDLPVSEK 4)TATESFASDPILYRPVAVALDTK | 15q22CM | Ubiquitously expressed |
| Sorbitol Dehydrogenase SORD | 1)LENYPIPEPGPNEVLLR | 15q15.3cM | Ubiquitously expressed (spermatogenic cells) |
| Lactate Dehydrogenase LDHA | 1)DYNVTANSK 2)VTLTSEEEAR 3)VIGSGCNLDSAR 4)LVIITAGAR 5)SADTLWGIQK 6)DQLIYNLLKEEQTPQNK 7)LKGEMMDLQHGSFLR 8)DLADELALVDVIEDK | 11p15.4 | Ubiquitous |
| Triosephosphate Isomerase 1 TPI1 | 1)TATPQQAQEVHEK 2)LDEREAGITEK 3)IAVAAQNCYK 4)SNVSDAVAQSTR 5)IIYGGSVTGATCK 6)VTNGAFTGEISPGMIK 7)HVFGESEDELIGQK 8)FFVGGNWK 9)DCGATWVVLGHSER 10)VPADTEVVCAPPTAYIDFAR 11)VVLAYEPVWAIGTGK 12)QSLGELIGTLNAAK 13)KFFVGGNWK 14)RHVFGESEDELIGQK 15)KQSLGELIGTLNAAK 16)VAHALAEGLGVIACIGEK | 12p13 | Ubiquitously expressed |

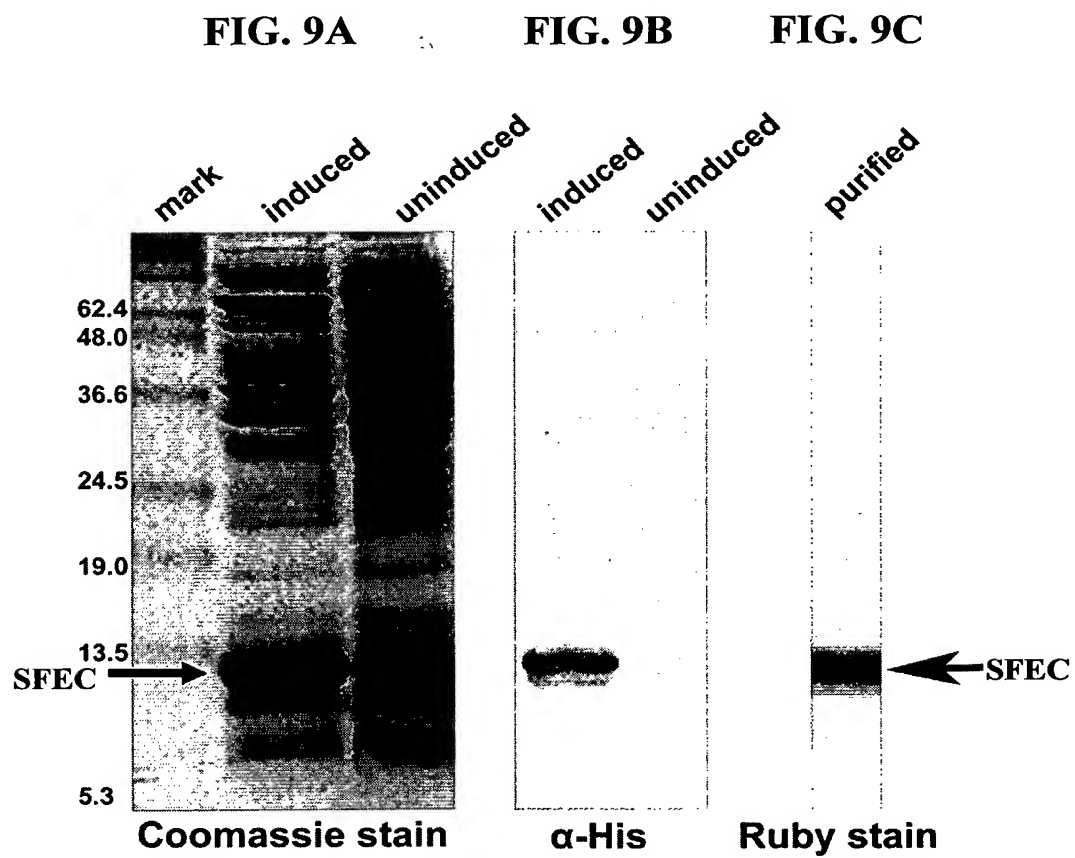
Figure 9- Expression and Purification of recombinant SFEC

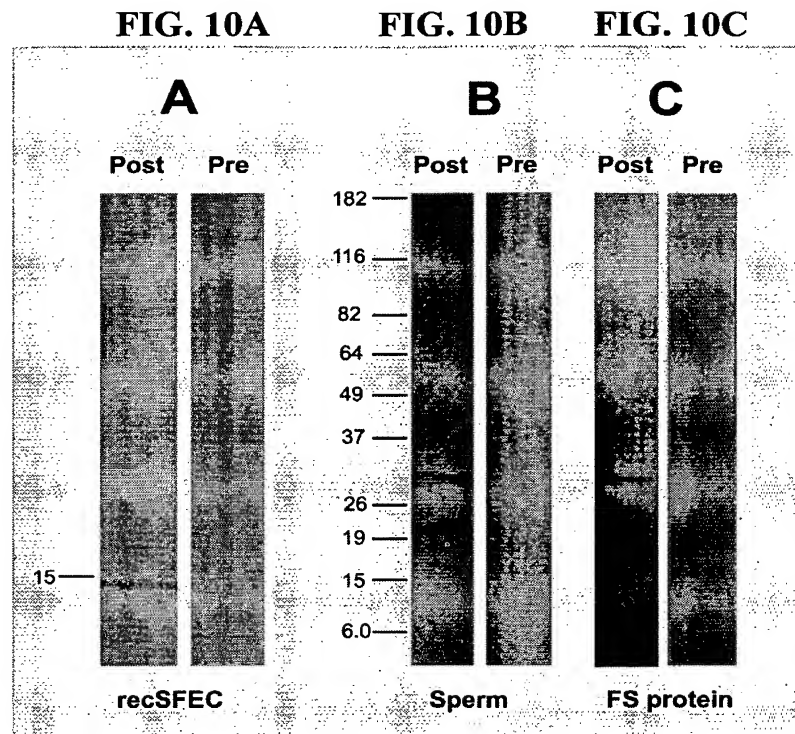
Figure 10- Western Analysis of SFEC

Figure 11- Localization of SFEC to the Principal Piece of the Flagellum

